EFFECT OF NITROGEN FROM DIFFERENT SOURCES ON THE GROWTH AND BIOMASS PRODUCTION OF *Spirulina platensis* (GOMONT) GEITLER

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Abstract: *Spirulina platensis* is a filamentous cyanobacterium known for its promising nutritional value used in several industries. The biomass produced after 24-days cultivation treated with sodium nitrate is significantly higher ($0.001 < \alpha_{0.05}$) than the cultures treated with urea. Cultures given with nitrogen concentration of 1.648 g/L of sodium nitrate had the highest optical density (5.895 ± 1.095) and dried biomass (2.265 ± 0.390 mg/mL) but not significantly higher with other sodium nitrate treatments (OD: $0.395 > \alpha_{0.05}$, DBM: $0.629 > \alpha_{0.05}$). The findings suggest that urea is not recommended as an alternative nitrogen source for *Spirulina.* Results also showed that lower nitrogen concentrations (0.103 g/L) of sodium nitrate can be used to cultivate *Spirulina* without compromising the biomass production. Moreover, this study showed that the peak of growth rate happens during the 16th day of cultivation.

Keywords: *Spirulina platensis, nitrogen sources, biomass production*

1. INTRODUCTION

One of the challenges in cultivating microalgae is its high production cost with a lower yield (Meseck et al., 2005). Since nutrition condition is an important facet in biomass production and its biochemical composition, cheaper growth media must be utilized (Markou et al., 2014). Among the nutrient constituents of growth medium, nitrogen comprises 10% of the total content (Soletto et al., 2005). Nitrogen is considered one of the most critical nutrients for growth, since it is a constituent in all structural and functional proteins such as peptides, enzymes, chlorophylls, energy transfer molecules, and genetic materials in algal cells (Cai et al., 2013; Hu, 2013). Thus, it is important to use an alternative nitrogen source that is commercially available. Among the available nitrogen sources, urea $CO(NH_2)_2$ is cheaper containing two nitrogen atoms in a single molecule with 46% of nitrogen (Danesi et al., 2002). Thus, it is required only in smaller amounts compared to sodium nitrate which is the known nitrogen source of growth media. Since the favorable nitrogen source for growth varies from species to species, it is inevitable to compare nitrogen sources and select the most appropriate to maximize the productivity of *Spirulina platensis*. This study compares the efficiency of urea and sodium nitrate as nitrogen sources as well as the influence of varying nitrogen concentration in the biomass production of *S. platensis*.

2. METHODOLOGY

2.1 Cultivation of Spirulina

S. platensis was cultured in Phycology Laboratory of the Research Institute for Science and Technology (RIST) in the Polytechnic University of the Philippines. Cultivation of *S. platensis* was conducted for 24 days using 800 mL culture bottles with Zarrouk's medium with an initial optical density of 0.3. The media was modified with varying nitrogen concentrations from sodium nitrate and urea as nitrogen sources and presented in Table 1. The cultures were maintained under laboratory condition with 24 hours illumination and continuous aeration at ambient temperature.

Table 1. Nitrogen concentration and the corresponding amount of nitrogen source present in the medium for the different experimental treatments.

Treatments	Nitrogen Concentration (g/L)	Sodium Nitrate (g/L)	Urea (g/L)
C1	0.103	0.625	0.224
C2	0.206	1.250	0.448
C3	0.412	2.500	0.896
C4	0.824	5.000	1.792
C5	1.648	10.000	3.584

2.2 Growth Analysis & Biomass Production

To determine the influence of nitrogen sources to the different culture conditions on the productivity of *S. platensis,* the pH, optical density (OD) and dried biomass of each culture were monitored every two days. The growth parameters are only limited to OD and dried biomass. In monitoring optical density of the culture, 1-cm cuvette was filled with 4 mL of the sample collected from each vessel and 4 mL of the Zarrouk's medium in a separate cuvette as the blank. The optical density of the samples were determined by reading at an absorbance of 622 nm using UV-Vis Spectrophotometer 1900. On the other hand, dried biomass was monitored by harvesting 10 mL of each culture by vacuum filtration. The harvested *S. platensis* was then dried and subsequently measured using the analytical balance.

3. RESULTS AND DISCUSSION

3.1 pH of cultures

The monitoring of pH was conducted daily for 24 days. The resulting pH for each treatment was summarized in Table 2. The pH of the experimental cultures ranges from 10.82 to 10.86. One-way analysis of variance (ANOVA) showed that pH of the cultures has no significant difference ($p > 0.05$) in all treatments. This implies that any changes in the biomass production and growth rate is not due to the changes in pH but due to the variables given to each culture such as the varying nitrogen concentrations from different sources.

Table 2. Summary of the pH from the various culture conditions throughout the 24-day cultivation period (SN = Sodium nitrate, U = Urea).

Treatments		**Mean ± SE**
Sodium Nitrate	Concentration (g/L)	
	0.103	10.85 ± 0.03^{a}
	0.206	10.86 ± 0.05^{a}
	0.412	10.83 ± 0.07^{a}
	0.824	10.85 ± 0.05^{a}
	1.648	10.82 ± 0.09^{a}
Urea		
	0.103	10.83 ± 0.07^{a}
	0.206	10.82 ± 0.05^{a}
	0.412	10.86 ± 0.09^{a}
	0.824	10.82 ± 0.04^{a}
	1.648	10.84 ± 0.09^{a}

*values in each column with similar superscript are not significantly different at $\alpha = 0.05$

The resulting pH for all the cultures ranges from 10.82 to 10.86 are within the range of optimal pH condition (> 7.5) to support *Spirulina* growth. Since *Spirulina* thrives in alkaline waters, it is important to supplement its artificial medium with elevated alkalinity. Culture medium with pH higher than 7.0 is considered alkaline and considered as the condition that inhibit the contamination of microalgae in an artificial medium (Ndjoundo et al., 2017).

3.2 Optical density and dried biomass

The biomass production of *Spirulina* was determined by the optical density (OD) and dried biomass (DB) of the cultures. Figures 1 and 2 summarize the influence of varying nitrogen concentrations from different nitrogen sources in the optical density and dried biomass of the cultures.

The OD (0.313 ± 0.003) at the start of cultivation period (Day 0) are not significantly different ($\alpha_{0.05} < 0.111$) among the various culture treatments. However, as the cultures continue to proliferate, the effect of nitrogen sources (sodium nitrate and urea) on the growth of *S. platensis* becomes very evident. Figure 1 shows that the cultures treated with sodium nitrate had increasing optical densities until the end of the cultivation period. On the other hand, the optical density of the cultures treated with urea started to decrease on the 16th day, which also exhibit discoloration and foul smell of ammonia on the 13th day.

Moreover, the resulting DB harvested (Figure 2) from the cultures had the same trend and showed no significant differences ($\alpha_{0.05} < 0.560$) among treatments. Throughout the cultivation period, cultures treated with sodium nitrate continued to increase in DB while those treated with urea started to decrease on the 20th day.

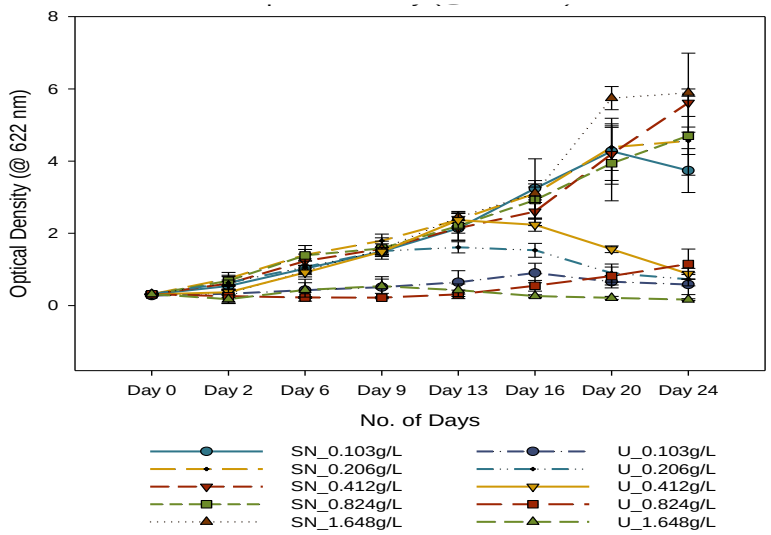

Figure 1. Optical density (OD) of *S. platensis* supplemented with different concentrations of nitrogen sources throughout the cultivation (SN = Sodium nitrate, U = Urea) with 0.3 as initial OD.

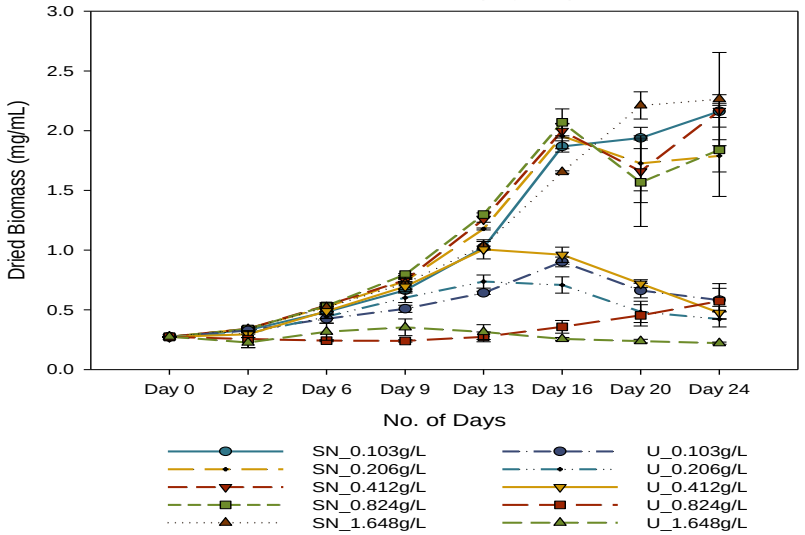

Figure 2. Dried biomass (mg/mL) of *S. platensis* supplemented with different concentrations of nitrogen sources throughout the cultivation (SN = Sodium nitrate, U = Urea) with 0.3 as initial OD.

It is also interesting to note that at the end of cultivation period, cultures treated with sodium nitrate showed significant difference ($\alpha_{0.05} > 0.001$) with cultures treated with urea as measured from the algal OD and DB. Among all the cultures treated with sodium nitrate, cultures treated with 1.648 g/L of nitrogen showed the highest OD (5.895 ± 1.095) and highest DB (2.265 ± 0.390 mg/mL) at the end of cultivation period. However, Kruskal-Wallis test shows that cultures treated with sodium nitrate have no significant difference with each other (OD: $\alpha_{0.05} > 0.395$; DBM: $\alpha_{0.05} > 0.629$).

The growth of *Spirulina* is dependent on the nutrient availability in the culture medium (Cornet et al., 1992). The concentration of nitrogen in culture medium affects both cell growth rate and biochemical compositions of microalgae (Wang et al., 2013). There are studies suggesting that nitrogen limitation in a culture medium slows down cell growth rate (Ho et al., 2014). Most microalgae can utilize various forms of nitrogen, including nitrate, nitrite, ammonium, and organic nitrogen sources such as urea (Becker, 1994). Each nitrogen source is first reduced to the ammonium form and assimilated into amino acids through a variety of pathways (Cai et al., 2013).

The response of *S. platensis* in varied nitrogen sources were measured by OD and DB of the cultures. Results show that the productivity of cultures treated with sodium nitrate is better than cultures treated with urea suggesting that the former is still more efficient to utilize in growing Spirulina cultures. The algal cells grown with urea collapsed during the cultivation period. Studies show that volatilization of urea occurs at higher pH level. Since urea is an organic form of nitrogen, it is easily hydrolyzed into ammonia due to high alkaline condition. At high pH, ammonia is capable of diffusing across the microorganism's cell membrane causing detrimental effect on microalgae by uncoupling photosynthesis (Soletto et al., 2005). In some cases, when ammonia volatilizes, it results in a net loss of nitrogen from the artificial medium (Killpack & Buchholz, 1993). Thus, it greatly affects the productivity of *Spirulina* since it is a constituent in the structural composition of cells and functional proteins such as enzymes in algal cells (Cai et al., 2013; Hu, 2013).

The highest nitrogen concentration (1.648 g/L) from sodium nitrate gave the highest value for OD (5.895 ± 1.095) and DB (2.265 ± 0.390 mg/mL). However, this treatment showed no significant difference with the results obtained using the lowest nitrogen concentration (0.103 g/L). This implies that *S. plantesis* can efficiently utilize sodium nitrate at concentrations between 0.103 g/L to 1.648 g/L. This suggests that Zarrouk's medium with 0.103 g/L nitrogen concentration can be used without compensating the algal biomass production. This modification enable cost-efficient medium and decrease the production cost.

3.3 Growth rate

The DB *S. plantesis* was translated to visualize the growth rate during the 24 days cultivation period (Figure 3). Generally, cultures do not have lag phase which already recorded an increased growth rate starting on Day 2. However, the growth rate of cultures treated with urea having 0.824 g/L and 1.648 g/L concentrations of nitrogen declined on Day 2 and showed fluctuating growth rate throughout the cultivation period. Most cultures recorded longer exponential phase starting on Day 6 until Day 16 recording the peak of growth rate at Day 16. During the peak, among the cultures treated with sodium nitrate and

urea, the highest growth rate was obtained by cultures having nitrogen concentrations of 0.824 g/L (0.126 ± 0.003 mg/mL) and 0.412 g/L (0.078 ± 0.004 mg/mL), respectively. Mann-Whitney U test showed that these two treatments have a significant difference with each other ($0.05 = \alpha_{0.05}$). On the other hand, One-way ANOVA showed that all cultures treated with sodium nitrate showed no significant difference with each other ($0.392 > \alpha_{0.05}$) during the peak. Moreover, no definite stationary phase was observed from all cultures since decline phase already started at Day 20.

It was observed that there is no lag phase in the growth curves of *S. plantesis* cultures. This might be due to the initial acclimatization, allowing the *S. plantesis* to adjust in varying concentrations. On the other hand, cultures with higher nitrogen concentrations of urea (> 0.824 g/L) had declining curve at the onset of cultivation. This support on the claim that *S. plantesis* cultures supplemented with urea decreases in productivity when exposed to high pH (Cai et al., 2013; Hu, 2013).

Result in growth rate also shows that there is prolonged exponential phase observed in cultures treated with sodium nitrate showing the peak at Day 16, suggesting the preference of *S. plantesis* in utilization of nitrogen from sodium nitrate. Spirulina transported the materials through passive diffusion (Boussiba, 1990; Rodriguez et al., 1994), which allows more efficient consumption of nitrogen, thus, facilitates exponential growth.

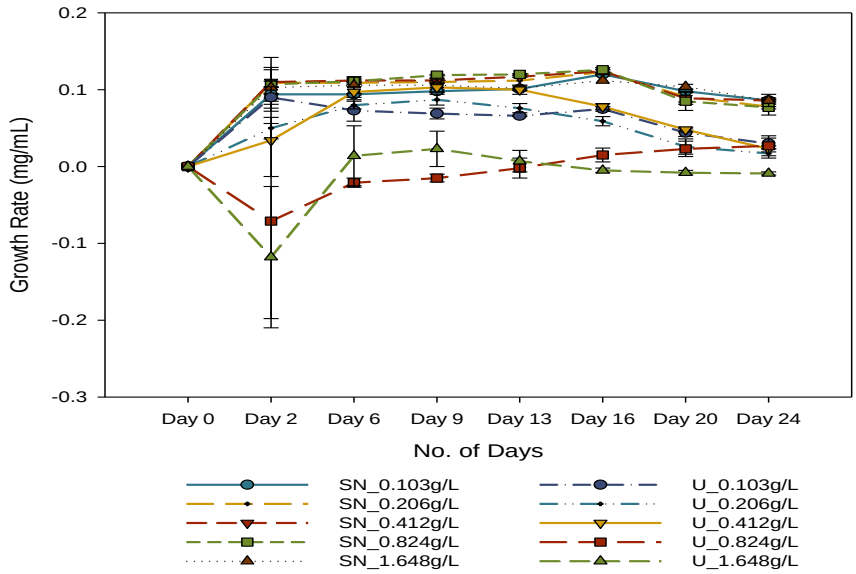

Figure 3. Growth rate (mg/mL) of *S. platensis* supplemented with different concentrations of nitrogen sources throughout the cultivation (SN = Sodium nitrate, U = Urea) with initial OD of 0.3.

During the 20th day, the growth rate started to decline. At this period, nutrients have been consumed by the *S. plantesis.* During this phase, conditions become unfavorable for the algal growth due to nutrient limitation that inhibits microalgae to undergo physiological processes (Wang et al., 2013).

4. CONCLUSIONS

S. platensis efficiently utilized sodium nitrate as source of nitrogen as compared to commercially available urea for biomass production. Thus, urea is not recommended as an alternative nitrogen source for the cultivation of *S. plantesis.* Nitrogen at concentration of 0.103 g/L from sodium nitrate is suggested to use in obtaining high biomass production. The best time to harvest biomass of *S. plantesis* at this concentration is between Day 16 to Day 24.

5. ACKNOWLEDGMENT

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